AD________________

Award Number: DAMD17-99-1-9395

TITLE: Tropomyosin-1, A Novel Class II Tumor Suppressor and a Biomarker of Human Breast Cancer

PRINCIPAL INVESTIGATOR: Gaddamanugu L. Prasad, Ph.D.

CONTRACTING ORGANIZATION: Wake Forest University
School of Medicine
Winston-Salem, North Carolina 27157

REPORT DATE: September 2001

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 2001	Annual (15 Aug 00 - 14 Aug 01)

4. TITLE AND SUBTITLE
Tropomyosin-1, A Novel Class II Tumor Suppressor and a Biomarker of Human Breast Cancer

5. FUNDING NUMBERS
DAMD17-99-1-9395

6. AUTHOR(S)
Gaddamanugu L. Prasad, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Wake Forest University School of Medicine
Winston-Salem, North Carolina 27157

E-Mail: gprasad@wfubmc.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
Previous research from this laboratory indicated that 1. the expression of Tropomyosin-1 (TM1), a microfilament-associated protein, is abolished from many human breast carcinoma cells, and; 2. that TM1 is suppressor of the malignant transformation. These data led to the hypothesis that TM1 plays an important role in mammary carcinogenesis. Therefore, we investigated whether TM1 could serve as a biomarker of breast cancer, and TM1 could function as a suppressor of the malignant growth of breast cancer cells. Initial experiments with a TM1-specific antibody suggested that TM1 expression may be absent in the breast tumors (Objective 1). We demonstrated that restoration of TM1 expression is adequate to suppress the malignant growth phenotype of MCF-7 cells, indicating that TM1 is a suppressor of the transformed growth (Objective 2). The reverse experiment, i.e., antisense-suppression of TM1 expression in normal mammary epithelial cells is in progress. MCF10A cells were transduced with TM1 antisense cDNA and those clones are being evaluated (Objective 3). To assess the structure-function relationship of tumor suppression by TM1, chimeras of TM1 and TM2 (which is not a tumor suppressor) are being constructed. These chimeras will be transfected into MCF-7 cells (Objective 4).

14. SUBJECT TERMS
Tropomyosin, biomarker, tumor suppressor, diagnosis/prognosis

15. NUMBER OF PAGES
39

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Introduction

Normal cell morphology is maintained and regulated by actin microfilaments. The presence of disorganized or poorly structured microfilaments is a prominent feature of many transformed cells. Downregulation of microfilament-associated proteins, such as tropomyosins (TMs) is hypothesized to result in the formation of functionally aberrant microfilaments, thus contributing to the manifestation of the malignant cells. Previous studies from this laboratory have identified that: 1). Tropomyosin-1 (TM1) is a suppressor of the transformed phenotype, and; 2). TM1 is consistently abolished in a large number of breast carcinoma cells that are tested. The main objectives of the proposed research are to assess the expression of TM1 in the tissue specimens of breast cancer patients and to investigate whether TM1 functions as a suppressor of the malignant growth of breast cancer.

Annual Summary

Technical Objective 1: Analysis of TM1 expression in human malignant breast tumors and benign lesions, and normal breast tissues:

Normal mammary epithelial cells elaborate multiple isoforms of TMs (Bhattacharya et al., 1990) . The spontaneously transformed breast cancer cells were shown to exhibit severe deficiency in TM expression, with the expression of several TMs is either downregulated or completely abolished. For example, TM38 protein is expressed in MDA MB231 cells, but found to be absent in MCF-7 cells. Expression of TM1, however, was consistently lacking in all the breast carcinoma cell lines tested, indicating that TM1 suppression could be a common event during mammary carcinogenesis. Based on these data, we hypothesized that loss of TM1 is a critical biochemical change in the malignant transformation of breast epithelial cells and that TM1 could be used as a novel biomarker of breast cancer.

We have proposed to utilize a TM1-specific antibody that was generated in our lab for assessing TM1 expression in normal and malignant breast tissues. Since multiple TMs are present in epithelial cells, and the presence of stromal components which abundantly express TM1, we chose to analyze the tissues by immunohistochemistry. The smooth muscle cells of the blood vessels also express TM1. Currently available antisera recognize multiple isoforms of TMs and, thus, do not permit accurate analysis of TM1 expression. Therefore, we developed a TM1-specific antibody in this lab. A 20 amino acid sequence of TM1, which is distinctive of this isoform, was used as an immunogen and antiserum was raised against it. The resulting antibody, designated as TM-20, and tested in immunoblotting for its specificity Details of the antibody are presented in the manuscript (appendix).

We have tested the utility of these reagents in immunoblotting using cell lines with known TM expression profiles. For example, normal MCF-10A cells express all TM isoforms, including TM1 and TM38 which co-migrate on SDS-polyacrylamide gels. In MCF-7 breast carcinoma cells, neither of these proteins are expressed, while MDA MB231 cells express TM38, but not TM1. When TM1-specific antibodies were used in immunoblotting, only MCF10A cells showed reactivity, attesting to the specificity of the reagents.

We have used one such antibody, TM20, for immunohistochemical analysis. In the initial experiments, we found that TM20 showed positive staining with normal mammary epithelium, while the malignant ducts lacked/reduced reactivity. This antibody will be used for larger scale screening experiments at the Wake Forest University School of Medicine.

In the event that we encounter any problems with TM20 reagent in screening, we will use other antibodies available generated in this laboratory or develop RNA–based detection methods such as *in situ* hybridization or Laser Capture Microscopy-RT-PCR methods (Kuecker et al., 1999). For that purpose, we started the collection of fresh tissue immediately after the surgery to ensure good quality of RNA in the tissues. This objective will be accomplished before the end of the grant period as proposed. Another alternative would be to assess the methylation status of TM1 promoter, since the emerging work from this laboratory indicates that the TM1 gene is methylated in breast cancer cells.

Technical Objective 2: Effects of expression of TM1 in human breast carcinoma cell lines and in normal mammary epithelial cell lines.

Our previous studies with experimentally transformed murine fibroblasts have demonstrated that TM1 is a suppressor of the malignant transformation, and that TM1 is a class II tumor suppressor (Braverman et al., 1996; Prasad et al., 1999). To examine the role of TM1 in mammary carcinogenesis, and to determine whether TM1 can suppress the malignant growth of a spontaneously transformed human breast carcinoma cells, the following experiments were carried out. These data are communicated for publication, and the manuscript is attached in the appendix. Therefore, the results are briefly presented below.

MCF-7 cells, which lack TM1 protein, were transduced with a recombinant retrovirus expressing TM1. Individual cell lines expressing TM1 were isolated and tested for the effects of TM1 expression on the morphology and growth properties. Restoration of TM1 expression resulted in the formation of tighter colonies with a more branched, tubular appearance. The presence of TM1 containing microfilaments are readily detected. TM1 expression significantly decreased the growth rates, compared to parental MCF-7 cells. A more profound effect was observed on the anchorage-independent growth property, which is a hall mark of the neoplastic phenotype. TM1 expression completely abolished the anchorage independent growth of MCF-7 cells, indicating that TM1 suppressed the malignant growth properties. It should be noted that the revertant cells remain sensitive to the growth control by estrogen.

Initial investigations into the possible mechanisms of TM1 expression suggested that c-myc levels are lower in the revertant cells compared to MCF-7 cells. Furthermore, investigation of E-cadherin complexes revealed that no changes in the total expression of E-cadherin and catenins. However, it was found that E-cadherin and the catenins are more tightly associated with cytoskeleton of the revertants. Currently, we are investigating the role of TM1 in anchoring E-cadherin/catenin complex to the cytoskeleton.

With these experiments, a majority of the goals of this Objective are accomplished, and we completed the Objective 2, ahead of the schedule, indicated in the Statement of Work. Additional experiments in progress are directed at elucidating the mechanism of tumor suppression by TM1. These include studies on utilization of TM1, expression of TM1 in MCF10A cells, expression of TGFα and amphiregulin.

3. Induction of transformed phenotype by repression of TM1 of TM1 expression: In order to test whether the loss of TM1 expression could lead to the expression of malignant transformation of mammary epithelium, antisense suppression of TM1 is proposed. TM1 was subcloned in antisense direction in the retroviral vector pBNC and antisense packaging cells of PA317 are generated. The MCF10A cells have been transduced with the recombinant pBNC retrovirus. Transduced cells were selected for resistance to G418 and single cell clones were selected. These cell lines are now being tested for TM1 expression and their ability to grow under anchorage independent conditions. The work is progressing as per the Statement of Work.

At this point we are considering more effective and novel strategies for antisense suppression. An alternative is the cre-lox mediated generation of TM1 knock out cell lines of normal mammary epithelial cells.

4. Structure-function relationship of TM1-mediated tumor suppression: Work on creating chimeras of TM1 (a tumor suppressor) and TM2 (not a tumor suppressor) has been initiated. We have completed the site directed mutagenesis to introduce a silent mutation to create a HindIII restriction site. This was accomplished by PCR and the resultant variants of TM1 and TM2, designated as 'TM1-h' and 'TM2-h' respectively, containing the HindIII site were sequenced. Switching of the carboxy (at AvaI) site and the central exons (HindIII-AvaI) is now in progress. We anticipate that the constructs will be ready for expression in MCF-7 cells soon. This work progressing according to the Statement of Work.

We propose to transfect the MCF- cells to identify the domains of TM1 that are responsible for tumor suppression. This will be a variation from the Statement of Work, where we proposed to carry out baculoviral expression and purification after the constructs are cloned. This modification will allow us to immediately assess the effects of the chimeric TMs on the growth phenotype of MCF-7 cells.

At this point, in parallel, we have chosen to test the use of epitope-tagged TMs in a different cell system. Several investigators have used epitope-tagged constructs to monitor the transfected genes. Epitope tagging of TM1 is expected to greatly facilitate the analysis of transfected TMs. To confirm that epitope tagging does not impair the tumor suppressive function of TM1, we chose to test the constructs in DT cell (ras-transformed NIH3T3 cells) system. Our previous published studies showed that DT cells are suitable models to test the ability of TM1 to reorganize the cytoskeleton and tumor suppression. A HA epitope was engineered to the amino terminus of TM1. The ATG initiation codon of TM1 was replaced with an oligonucleotide sequence encoding HA epitope. The recombinant TM1 was subcloned into a eukaryotic expression vector, and transfected into DT cells. Detailed characterization of the transfected cells is in progress. Depending on those results, we will either continue to work with the constructs that are generated, or employ the epitope-tagged constructs for easy monitoring in the transfection experiments.

Key Research accomplishments

- Several TM1-specific antisera have been developed and their specificity is tested.
- Screening of breast tissues for TM1 expression is in progress.
- In addition to originally proposed immunohistochemical screening, we are now employing *in situ* hybridization and Laser Capture microscopy-RT PCR strategy. Conditions for in situ hybridization are standardized, and sample collection is in progress. We are now determining the conditions for quantifying the RT-PCR.
- Restoration of TM1 expression reverts MCF-7 cells.
- The revertant MCF-7 cells display improved cell-cell adhesion complexes, with e-cadherin and β-catenin associated more tightly to the cell-cell junctions.

Reportable Outcomes

Manuscripts and Abstracts

1. Manuscripts:
   1.1. Abolition of Tropomyosin-1 gene expression in breast cancer cells by methylation and histone deacetylation. Shantaram Bharadwaj and G. L. Prasad (manuscript in preparation).
   1.2. Suppression of transformed phenotype of breast cancer by tropomyosin-1. Kalyankar Mahadev, Gira Raval, Mark Willingham, Barbara Vonderhaar, David Salomon and G. L. Prasad (manuscript communicated).
2. Two abstracts were presented:
   2.1. Tropomyosin-1 is a suppressor of the malignant phenotype of breast cancer cells. (2001) G. L. Prasad S. Bharadwaj, G. N. Raval, and B. K. Vonderhaar. Gordon conference on Mammary Gland Biology.

The U.S. Army Medical Research and Materiel Command under DAMD-99-1-9395 supported this work.

Conclusions

In summary, the major accomplishment of our work for this year is to demonstrate that TM1 indeed is a tumor suppressor of breast carcinoma cells. We showed that restoration of TM1 expression in MCF-7 model cell system, abolishes anchorage-independent growth, significantly decreases the growth rates, while not altering the estrogen growth controls. We also found that TM1 might mediate these effects through E-cadherin-βcatenin pathway (Polakis, 1837; Yap et al., 1997).

These findings could have important implications in understanding biology of breast cancer and possibly exploring novel therapies. First of all, our studies indicate that the assembly of cell-cell adhesion junctions can be modulated by changes in microfilament associated proteins. This suggests that microfilament proteins through their ability to regulate the assembly of cadherin-catenin junctions could, in fact, alter the gene expression via TCF-LEF/-βcatenin pathway (Barker et al., 2000). This possibility sheds light on the TM1-mediated suppression of the revertant phenotype, which is being investigated.

A second implication is to test whether TM1 can be used for gene therapy of breast cancer. Since TM1 appears to be a transformation-specific suppressor, it is an attractive therapeutic target. We are planning to explore this possibility by employing adenoviral vectors for the gene delivery using Adeasy adenoviral vectors (He et al., 1998). We have generated the recombinant adenoviral vector designed to express TM1. Additionally, we will use the adenoviral expression system as an inducible vector to investigate the early events in tumor suppression by TM1.

References

Barker, N., Morin, P. J., and Clevers, H. (2000). The Yin-Yang of TCF/beta-catenin signaling. Advances in Cancer Research *77*, 1-24.

Bhattacharya, B., Prasad, G. L., Valverius, E. M., Salomon, D. S., and Cooper, H. L. (1990). Tropomyosins of human mammary epithelial cells: consistent defects of expression in mammary carcinoma cell lines. Cancer Research *50*, 2105-12.

Braverman, R. H., Cooper, H. L., Lee, H. S., and Prasad, G. L. (1996). Anti-oncogenic effects of tropomyosin: isoform specificity and importance of protein coding sequences. Oncogene *13*, 537-45.

He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. (1998). A simplified system for generating recombinant adenoviruses. Proceedings of the National Academy of Sciences of the United States of America *95*, 2509-14.

Kuecker, S. J., Jin, L., Kulig, E., Oudaogo, G. L., Roche, P. C., and Llyod, R. V. (1999). Analysis of PRL, PRL-R, TGFb1, and TGFb-RII gene expression after Laser Capture Microdissection. Applied immunohitochemistry & Molecular Morphology *7*, 193-200.

Polakis, P. (1837). Wnt signaling and cancer. Genes & Development *14*, 1837-51.

Prasad, G. L., Masuelli, L., Raj, M. H., and Harindranath, N. (1999). Suppression of src-induced transformed phenotype by expression of tropomyosin-1. Oncogene *18*, 2027-31.

Yap, A. S., Brieher, W. M., and Gumbiner, B. M. (1997). Molecular and functional analysis of cadherin-based adherens junctions. Annual Review of Cell & Developmental Biology *13*, 119-46.

APPENDIX

1. A manuscript: Suppression of the transformed phenotype of breast cancer by tropomyosin-1.
2. A meeting abstract submitted at the Gordon Conference on Mammary gland Biology, June 3-8, 2001.

Suppression of the transformed phenotype of breast cancer by tropomyosin-1.

Kalyankar Mahadev[§], Gira Raval, Mark C. Willingham, Barbara Vonderhaar[¶], David Salomon[¶] and G. L. Prasad*.

Departments of General Surgery and Cancer Biology, Department of Pathology Wake Forest University School of Medicine, Winston-Salem, NC 27157; [¶]Basic Research Laboratory, Center for Cancer Research, NCI, NIH, Bethesda, MD 20892-1402.

Key words: cytoskeleton, tropomyosin, breast cancer, E-cadherin, β-catenin

*Correspondence: G. L. Prasad, 336-716-2788 (Telephone); 336-716-2528. email: gprasad@wfubmc.edu.

Total number of characters: 27, 274.

Abstract

Changes in the expression of microfilament-associated proteins such as tropomyosins (TMs), are associated with the transformed phenotype. Our previous work demonstrated: 1. the loss of expression of tropomyosin-1 (TM1) is a common biochemical event found in many malignantly transformed cells, and; 2. TM1 is a suppressor of the malignant phenotype induced by retroviral oncogenes. We also showed that TM1 expression.is consistently abolished in the human breast carcinoma cell lines. In this work we have tested the hypothesis that TM1 is a tumor suppressor of breast cancer.

MCF-7 cells, which have lost the expression of TM1, were utilized as a model of human breast cancer. Restoration of TM1 expression in MCF-7 cells (MCF-7/T) resulted in slower growth rate, but remain sensitive to growth control by estrogen. More significantly, MCF-7/T cells failed to grow under anchorage-independent conditions. TM1 re-expression alters the interaction of E-cadherin-catenin complex with the cytoskeleton, indicating that TM1-induced microfilaments could play a significant role in suppression of the malignant phenotype. Thus, TM1 appears to be essential for normal growth and differentiation of mammary epithelium.

Introduction

During the neoplastic transformation cells accumulate several different mutations and undergo extensive changes in gene expression. While the etiology of the vast majority of tumors is unknown, it is generally accepted that multiple genetic events contribute to the neoplastic transformation of cells (Kinzler and Vogelstein, 1997). The mortality of cancer is primarily due to the ability of the neoplastic cells to invade and metastasize in tissues where the primary cells do not normally grow. Tumor metastasis involves multiple steps including the loss of normal growth controls, the derangement of cytoskeletal organization, and the capacity to become motile and invasive (Button *et al.*, 1995). Tumor cells also manifest altered cell-cell adhesion and abnormal microfilaments, which facilitate invasion (Bissell *et al.*, 1999; Sommers, 1996). Microfilaments are linked to both integrin and cadherin-catenin complexes which regulate cell-matrix and cell-cell adhesion respectively (Ben-Ze'ev, 1997).

Our work on the role of cytoskeletal proteins in cellular transformation has demonstrated that derangements in tropomyosin (TM) expression are a common biochemical change in many breast carcinoma cells (Bhattacharya *et al.*, 1990), and other transformed cells (Cooper *et al.*, 1987; Cooper *et al.*, 1985; Lin *et al.*, 1997; Matsumura *et al.*, 1983). Furthermore, we demonstrated, using oncogene-transformed murine fibroblasts, that restoration of TM1 expression is adequate to revert the malignant phenotype induced by functionally diverse oncogenes such as ras and src (Braverman *et al.*, 1996; Prasad *et al.*, 1993; Prasad *et al.*, 1999). TMs are a family of closely related actin-binding proteins (Lin *et al.*, 1997; Pittenger *et al.*, 1994). Multiple isoforms of TMs are expressed from four genes via alternate splicing in a highly tissue specific manner (Lin *et al.*, 1997; Pittenger *et al.*, 1994). TMs bind to actin filaments and stabilize them against the action of gel severing proteins such as gelsolin (Ishikawa *et al.*, 1989a; Ishikawa *et al.*, 1989b).

Although it is known that suppression of TMs is a prominent feature of many experimentally transformed murine cell lines, the relevance of TMs in human cancers is largely unknown. To that end, we have investigated the role of TMs in mammary carcinogenesis. In normal human mammary epithelial cells, 7 different isoforms of TMs are expressed (Bhattacharya *et al.*, 1990). Among these, TM1, TM2, TM3 and an epithelial specific species TM38, may be categorized as high M_r TMs. Isoforms TM32a, TM32b and another epithelial specific protein, TM32 are known to be low M_r TMs. In spontaneously transformed human breast carcinoma cell lines, loss of expression of multiple isoforms of TMs have been observed. More significantly, expression of TM1 is completely abolished in the transformed cell lines, suggesting that suppression of TM1 could be a pivotal event leading to the acquisition of the neoplastic phenotype by mammary epithelial cells.

The experimentally transformed fibroblasts employed in earlier studies to define a causal relationship of TMs to cell transformation are generally generated by a single well defined transforming oncogene (Prasad *et al.*, 1993; Prasad *et al.*, 1999). Most human cancers, on the other hand, originate in epithelial cells as a result of multiple genetic defects. Furthermore, mechanisms that lead to the neoplastic transformation of epithelial cells could be different and more complex. For example, while both Raf and Ras transform fibroblasts, epithelial cells are transformed only by Ras (Oldham *et al.*, 1996). Another complexity with epithelial cells is that, at least two more TM isoforms are expressed in epithelial cells compared to fibroblasts (Bhattacharya *et al.*, 1990). Thus, it remains to be established whether TM1 can function as a suppressor of the malignant phenotype of spontaneously transformed, human-derived carcinoma cells. To further investigate the role of TM1 in human cancers, we ascertained whether restoration of TM1 expression in the MCF-7 human breast cancer cell line has an effect on the growth and transformation of these cells.

Materials and Methods

Normal mammary epithelial MCF10A cells were obtained from Dr. Jose Russo, Fox Chase Cancer Center, Philadelphia (Prasad *et al.*, 1992) and MCF-7 cells were purchased from ATCC. DT/TM1 and DT/TM1-TM2 cells were fibroblast cell lines as previously described (Shah *et al.*, 2001; Shah *et al.*, 1998). Anti-TM polyclonal antiserum that recognizes TM1 as well as the other TMs is used for immunoblotting, immunocytochemistry and immunoprecipitations (Prasad *et al.*, 1999; Shah *et al.*, 1998). A 20 amino acid sequence (187-206) (Prasad *et al.*, 1991) was used to generate a TM1-specific antibody. It detects TM1, but not other TMs in immunoblotting.

For retroviral gene transfer of TM1 into MCF-7 cells, a pBNC recombinant virus was used as described (Prasad *et al.*, 1994; Prasad *et al.*, 1993), except that an amphitrophic packaging cell line PA317 was used to generate infectious virus. Clonal populations of TM1 expressing MCF-7 (MCF-7/T) cells and empty vector transdulced MCF-7 (MCF-7/V) cells were generated..

Growth rates: Growth rates of the cells were measured in monolayer. Briefly, 2 $\times 10^5$ cells were plated in normal (10%) serum. At regular intervals, cells were harvested and counted using a hemocytometer. Experiments involving estrogen deprivation and supplementation were performed phenol red-free basal medium. To test the effects of estrogen on the growth of the cells, 72h after plating, normal medium was replaced with a medium containing charcoal stripped FBS alone (minus estrogen), or with a supplementation of 100nM 17-β-estradiol (plus estrogen).

Immunofluorescence: Immunofluorescence was performed as described elsewhere (Shah *et al.*, 2001). The samples were mounted using Prolong Antifade kit (Molecular Probes) and viewed using a Zeiss confocal microscope with a 60X water objective. For determination of the intensity of staining, the samples were viewed with a Zeiss Axioplan 2 microscope using either a 63x oil objective. The images were captured using a Dage MTI camera (model 300) and IFG 310 controller. The samples were photographed using different gate settings, which allows accumulation different of numbers of frames. The gate setting is inversely linked to the brightness of staining. For example, if a gate setting of 4 is used to photograph, 4 different individual frames will be taken and integrated in a final image as a JPEG files. However, if a gate setting of 8 is required, it suggests that the image is about half as intensely stained as the first one. In these experiments, both the gain and black level, which affect the image quality, were unchanged.

Results and Discussion:

Restoration of TM1 expression in MCF-7 cells: Normal mammary as well as other epithelial cells express 7 different TMs, two more TMs than found in fibroblasts, that are readily detected in 2-D gels, but do not resolve in SDS PAGE (Bhattacharya *et al.*, 1990; Prasad *et al.*, 1991). One of these additional TMs, TM38, co-migrates with TM1 in SDS PAGE (Figure 1A, top panel). The other TM isoform, TM32, is not resolved from the two low M_r TMs. Expression of TM1 is consistently abolished in all the breast carcinoma cell lines tested, indicating that inhibition of TM1 synthesis is an important event during oncogenic transformation of mammary epithelial cells (Bhattacharya *et al.*, 1990); the expression of other TMs including that of TM38, however, varies. For example, in MDA MB231 and MDAMB453 cells TM38 expression is detectable by immunoblotting with polyclonal anti-TM antisera, and by 2-D gels (data not shown) (Bhattacharya *et al.*, 1990); in MCF-7 cells both TM1 and TM38 are lacking.

To facilitate the analysis of TM1 expression, we generated a specific anti-peptide antiserum. Two additional fibroblast cell lines used as positive controls for TM1 expression, DT/T3 and DT/TM1-TM2 cells express either TM1 alone or TM1 and TM2 together were previously described (Prasad *et al.*, 1993; Shah *et al.*, 1998); these cells lack TM38. Immunoblotting with TM1-specific antibody reveals the presence of TM1 in these fibroblast-derived cell lines (Figure 1A, middle panel). TM1 was lacking in MCF-7, MDA MB231 and MDA MB453 breast cancer cell lines; the latter two cell lines express TM38 as shown in the top panel. MCF-7 cells transduced to re-express TM1, designated MCF-7/T contain TM1 (described below). Thus, interpretation of immunoblotting results with TM antibodies warrants additional controls.

In MCF-10A human mammary epithelial cells, TM1 is expressed from a 1.1kb mRNA. In MCF-7 human breast carcinoma cells, expression of both TM1protien and its cognate mRNA is totally abolished (Figure 1B) (Bhattacharya *et al.*, 1990). We have analyzed three independent MCF-7/T cell lines, and two MCF-7/V cell lines along with the parental MCF-7 cells. Transduction of MCF-7 cells with TM1 cDNA resulted in the expression a 2.0kb mRNA from which TM1 is transcribed, which is absent in the control cells.

TM expression in MCF-7/T cells was analyzed by immunoblotting and two dimensional gel analyses (Figure 1A and Fig 1C). Among the muscle-type high M_r TMs that are present in epithelial cells, only TM3 is present in MCF-7 and MCF-7/V cells. As described above, TM1 is absent in the parental and vector control cells. MCF-7/T cells, on the other hand, synthesize abundant quantities of TM1 protein in all the cell lines tested. Transduced TM1 protein also enters the cytoskeletal compartment (data not shown).

Morphology of MCF-7/T cells: Restoration of TM1 expression in MCF-7 cells resulted in significant morphological changes. MCF-7 cells (Figure 2A top panel) and the vector control cells shows that they grow as rather loosely adhering clusters. MCF-7/T cells in general grow in tighter clusters and form distinctive tubular structures (Figure 2A, bottom panel).

Immunocytochemical staining with anti-TM antibody of parental MCF-7 cells showed weak staining with the TM antiserum although MCF-7 cells express at least one high Mr TM isoform and low M_r TMs (Figure 2B, panels A-C). In MCF-7/T cells, TM staining was intense and colocalizes with microfilaments (Figure 2B, panels D-F). Although TM staining was found throughout the cell body, it was brightest around the nucleus in MCF-7/T cells while a weak signal was obtained with MCF-7/ cells (Figure 2 E & B, respectively).

Growth Properties: The effect of TM1 expression on the growth properties of MCF-7 cells was assessed in monolayer cultures. MCF-7, a vector control cell line (V1), and three cell lines expressing TM1 (T1, T2 and T3) were used to measure the growth rates. Under normal serum conditions, the unmodified MCF-7 and those transduced with empty vector grew rapidly at nearly identical rates (Figure 3A). However, all the three individual cell lines expressing TM1 grew at comparable rates, but demonstrated strikingly slower growth rates than the parental MCF-7 or MCF-7/V cell lines. Thus, restoration of TM1 expression decreases the proliferation of breast carcinoma cells. This is consistent with the previous studies which have shown that TM1 decreases the growth of src-transformed fibroblasts (Prasad *et al.*, 1999)

Estrogen regulates the growth and differentiation status of MCF-7 cells. Since TM1 expression decreases the growth of these cells, we tested whether MCF-7/T cells remain sensitive to growth controls by estrogen (Figure 3 B & C). When cells were cultured in the absence of estrogen using charcoal stripped fetal bovine serum, growth of all the cell lines, including those expressing TM1, was inhibited by about 50%. Even under these conditions, MCF-7 and MCF-7/V1 (Figure 3B) cells maintained relatively higher growth rate than MCF-7/T cells (Figure 3C). Supplementation with 100nM βestradiol in charcoal-treated serum containing medium resulted in higher growth rates which was comparable to those seen with medium containing normal serum. In the presence of estrogen MCF-7 and MCF-7/V1 (Figure 3B) cells demonstrated profoundly enhanced growth compared to MCF-7/T cells (Figure 3C). Addition of 5-hydroxytamoxifen, inhibited the stimulatory effect of estrogen (data not shown). These data show that restoration of TM1 expression decreases the growth of MCF-7 cells, without altering the sensitivity to estrogen.

Anchorage independent growth: The proliferation of normal cells is tightly regulated by growth signals of integrin-extracellular matrix interactions, which is often deregulated in tumor cells (Schwartz, 1997). The ability of tumor cells to proliferate independent of adhesion closely correlates with tumorigenic potential, which is often assayed by anchorage-independent growth in soft agar. Previous studies have shown that TM1 is a suppressor of malignant transformation(Prasad *et al.*, 1993; Prasad *et al.*, 1999). The data presented in Figure 3 show that re-expression of TM1 decreases the growth of MCF-7 cells. Therefore, we examined whether TM1 inhibits anchorage-independent growth of MCF-7 cells. MCF-7, V1 and V2 cells grew rapidly and formed a large number of colonies within 2 weeks (Figure 4A and 4B). In contrast, the three clones of MCF-7 cells expressing TM1 did not grow in parallel cultures. These data demonstrate that re-expression of TM1 abolishes the malignant potential of breast carcinoma cells and support our earlier studies on the anti-transformation effects of TM1 (Prasad *et al.*, 1999).

Cell-cell adhesion molecules in the revertant breast cancer cells: E-cadherin, often referred to as a metastasis suppressor, is generally expressed either at low levels (Mareel *et al.*, 1997; Sommers, 1996) or is mutated in some breast cancer specimens (Huiping *et al.*, 1999). E-cadherin forms a multiprotein complex with α, β, and γ catenins is anchored to microfilaments, and is implicated in regulating tissue integrity, polarity and differentiation. Stable association of cadherin-catenin complex to microfilaments is considered as a requirement for normal functioning of cadherin complexes (Gumbiner, 2000; Yap *et al.*, 1997). Furthermore, free, soluble β-catenin, which is not associated with cadherins, is a key player in the wnt signaling pathways (Barker *et al.*, 2000; Polakis, 1837). Although wnt signaling pathways have been well characterized in other systems, it is unclear as to its significance in breast cancer. Since, cells expressing TM1 form tighter clusters and display a tubular morphology indicating enhanced differentiation, we investigated whether TM1-induced reversion of breast carcinoma cells involves changes in the expression of E-cadherin or the catenins.

Cytoplasmic lysates were prepared from actively growing cells and were immunoblotted with antibodies against E-cadherin and α-,β-, and γ-catenins. MCF-7 and the two vector control cell lines express similar levels of all the four proteins tested. The three revertant cell lines, however, showed significantly and consistently lower levels of E-cadherin and the catenins (Figure 5A). Since TM1 expression is associated with the re-emergence of microfilaments, we investigated a possibility that the cadherin complex may be more tightly associated with the cytoskeleton in the revertants, thus, forming stronger cell-cell junctions which may make them less soluble. To examine the total expression of these proteins, cells were extracted under more vigorous conditions, using RIPA buffer to lyse the cells, and tested for the presence of the components of cadherin-catenin complex. Under these conditions, no detectable differences in the expression of E-cadherin or the catenins were found between the transformed (MCF-7 and V) and the revertant TM1-expressing MCF-7/T clones (Figure 5B)

To investigate whether the localization of E-cadherin and the catenins is altered in the revertants, immunocytochemistry was performed. In MCF-7 and the revertant cells, E-cadherin and β-catenins were detectable at the cell-cell junctions. In MCF-7 cells, E-cadherin's presence was evident in the perinuclear area as well as in the cytoplasm (data not shown). Further analysis of the organization of cell adhesion molecules in the revertant cells revealed significant differences from the parental MCF-7 cells.

While both the parental MCF-7 and MCF-7/T cells contained E-cadherin and β-catenin at cell-cell junctions, their association with the detergent insoluble fraction was different. MCF-7 cells had significantly lower detergent-resistant E-cadherin and β-catenin at the cell-cell junctions (Figure 5C, panel a, b) vis-à-vis MCF-7/T cells (Figure 5C, panel c, d). The intensity of either E-cadherin or β-catenin at the cell-cell junctions was higher in MCF-7/T cells. MCF-7/T cells consistently retained higher amounts of E-cadherin and β-catenin at the cell-cell junctions than in MCF-7 cells. These data suggest that in the revertant cells, E-cadherin and β-catenin are more tightly linked to the cytoskeleton, which is in line with the immunoblotting results. However, under these conditions, α- and γ-catenins, were found to be at comparable levels in MCF-7 and MCF-7/T cells (data not shown).

Enhanced detergent solubility of E-cadherin and β-catenin is more frequently observed in transformed cells, which may result in the assembly of defective adhesion junctions. Consistent with these data, it was reported that ras-transformation of breast epithelial cells does not change the expression of E-cadherin or β-catenin, but results the increased detergent solubility of these molecules (Kinch *et al.*, 1995). It is likely that the improved/TM1-induced microfilaments may provide the necessary support for clustering and stability of the cadherins (Yap *et al.*, 1997).

Morphological and growth properties (Figures 2-4) of TM1 expressing cells support the thesis that TM1 is a suppressor of the malignant growth phenotype of breast cancer cells. Taken together with the previous work TM1 appears to be a general suppressor of cellular transformation, regardless of the initial transforming event. TM1, therefore, may be classified as a class II tumor suppressor. Inactivation of class II tumor suppressors may be necessary for acquiring the malignant phenotype, and this is likely to be a result of the inactivation of the class I tumor suppressors such as Rb or p53, or activation of cellular oncogenes (Sers *et al.*, 1997). The ability of TM1 to restore normal growth properties of human breast cancer cells underscores the importance of TM1, and the cytoskeleton in normal growth differentiation of human epithelia.

Vinculin, another microfilament-associated protein has been shown to substitute for α-catenin in cadherin-catenin complexes in a different breast carcinoma cell line, where α-catenin was mutated (Hazan *et al.*, 1997). Furthermore, upon activation of the EGF receptor, α-catenin and vinculin dissociate from E-cadherin-catenin complex suggesting that the EGF receptor regulates the linkage of microfilaments to adherens junctions (Hazan *et al.*, 1997). EGF receptor activation, however, does not alter the levels of either E-cadherin, or the catenins. Similarly, phosphorylation of cytoplasmic portion of E-cadherin by casein kinase II and GSK-3β enhances binding to β-catenin (Lickert *et al.*, 2000). Therefore, altered post-translational modifications such as phosphorylation, may be critical in facilitating a tighter linkage of cadherin-and the catenins to cytoskeleton in the TM1-mediated reversion of MCF-7 cells.

While the exact mechanisms of TM1-induced stabilization of cadherin-catenin complexes and suppression of the transformed phenotype of breast cancer cells are unclear at present, TM1-induced cytoskeletal reorganization appears to be mediated by Rho kinase (Shah *et al.*, 1998). It remains to be tested whether such pathways are critical for stabilization of cell signaling molecules. Given the multitude of roles of the actin cytoskeleton, it is likely that TM1-induced cytoskeleton will be critical in TM1-induced tumor suppression.

Acknowledgements: This work is supported by grants from the US Department of Defense Breast Cancer Program (CDA DAMD-98-1-8162; and, DAMD-991-9395) and American Cancer Society (RPG-99-069-01-CSM). We thank Ken Grant for help with confocal microscopy.

Footnote: tropomyosin, TM; tropomyosin-1, TM1.

References:

Barker, N., Morin, P. J. and Clevers, H. (2000). The Yin-Yang of TCF/beta-catenin signaling. *Adv. Cancer Res.* **77,** 1-24.

Ben-Ze'ev, A. (1997). Cytoskeletal and adhesion proteins as tumor suppressors. *Curr. Opin. Cell Biol.* **9,** 99-108.

Bhattacharya, B., Prasad, G. L., Valverius, E. M., Salomon, D. S. and Cooper, H. L. (1990). Tropomyosins of human mammary epithelial cells: consistent defects of expression in mammary carcinoma cell lines. *Cancer Res.* **50,** 2105-12.

Bissell, M. J., Weaver, V. M., Lelievre, S. A., Wang, F., Petersen, O. W. and Schmeichel, K. L. (1999). Tissue structure, nuclear organization, and gene expression in normal and malignant breast. *Cancer Res.* **59,** 1757-1763s; discussion 1763s-1764s.

Braverman, R. H., Cooper, H. L., Lee, H. S. and Prasad, G. L. (1996). Anti-oncogenic effects of tropomyosin: isoform specificity and importance of protein coding sequences. *Oncogene* **13,** 537-45.

Button, E., Shapland, C. and Lawson, D. (1995). Actin, its associated proteins and metastasis. *Cell Motility & the Cytoskeleton* **30,** 247-51.

Cooper, H. L., Bhattacharya, B., Bassin, R. H. and Salomon, D. S. (1987). Suppression of synthesis and utilization of tropomyosin in mouse and rat fibroblasts by transforming growth factor alpha: a pathway in oncogene action. *Cancer Res.* **47,** 4493-500.

Cooper, H. L., Feuerstein, N., Noda, M. and Bassin, R. H. (1985). Suppression of tropomyosin synthesis, a common biochemical feature of oncogenesis by structurally diverse retroviral oncogenes. *Mol. Cell. Biol.* **5,** 972-83.

Gumbiner, B. M. (2000). Regulation of cadherin adhesive activity. *J. Cell Biol.* **148,** 399-404.

Hazan, R. B., Kang, L., Roe, S., Borgen, P. I. and Rimm, D. L. (1997). Vinculin is associated with the E-cadherin adhesion complex. *J. Biol. Chem.* **272,** 32448-53.

Huiping, C., Sigurgeirsdottir, J. R., Jonasson, J. G., Eiriksdottir, G., Johannsdottir, J. T., Egilsson, V. and Ingvarsson, S. (1999). Chromosome alterations and E-cadherin gene mutations in human lobular breast cancer. *British Journal of Cancer* **81,** 1103-10.

Ishikawa, R., Yamashiro, S. and Matsumura, F. (1989a). Annealing of gelsolin-severed actin fragments by tropomyosin in the presence of Ca2+. Potentiation of the annealing process by caldesmon. *J. Biol. Chem.* **264,** 16764-70.

Ishikawa, R., Yamashiro, S. and Matsumura, F. (1989b). Differential modulation of actin-severing activity of gelsolin by multiple isoforms of cultured rat cell tropomyosin. Potentiation of protective ability of tropomyosins by 83-kDa nonmuscle caldesmon. *J. Biol. Chem.* **264,** 7490-7.

Kinch, M. S., Clark, G. J., Der, C. J. and Burridge, K. (1995). Tyrosine phosphorylation regulates the adhesions of ras-transformed breast epithelia. *J. Cell Biol.* **130,** 461-71.

Kinzler, K. W. and Vogelstein, B. (1997). Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* **386,** 761, 763.

Lickert, H., Bauer, A., Kemler, R. and Stappert, J. (2000). Casein kinase II phosphorylation of E-cadherin increases E-cadherin/beta-catenin interaction and strengthens cell-cell adhesion. *J. Biol. Chem.* **275,** 5090-5.

Lin, J. J., Warren, K. S., Wamboldt, D. D., Wang, T. and Lin, J. L. (1997). Tropomyosin isoforms in nonmuscle cells. *Intl. Rev. Cytol.* **170,** 1-38.

Mareel, M., Boterberg, T., Noe, V., Van Hoorde, L., Vermeulen, S., Bruyneel, E. and Bracke, M. (1997). E-cadherin/catenin/cytoskeleton complex: a regulator of cancer invasion. *J. Cell Phys.* **173,** 271-4.

Matsumura, F., Lin, J. J., Yamashiro-Matsumura, S., Thomas, G. P. and Topp, W. C. (1983). Differential expression of tropomyosin forms in the microfilaments isolated from normal and transformed rat cultured cells. *J. Biol. Chem.* **258,** 13954-64.

Oldham, S. M., Clark, G. J., Gangarosa, L. M., Coffey, R. J., Jr. and Der, C. J. (1996). Activation of the Raf-1/MAP kinase cascade is not sufficient for Ras transformation of RIE-1 epithelial cells. *Proc. Natl. Acad. Sci. USA.* **93,** 6924-8.

Pittenger, M. F., Kazzaz, J. A. and Helfman, D. M. (1994). Functional properties of non-muscle tropomyosin isoforms. *Curr. Opin. Cell Biol.* **6,** 96-104.

Polakis, P. (1837). Wnt signaling and cancer. *Genes & Dev.* **14,** 1837-51.

Prasad, G. L., E.M.Valverius, E.McDuffie and H.L.Cooper. (1992). cDNA cloning and expression of an epithelial cell protein, HME1, that is down regulated in neoplastic mammary cells. *Cell Growth. Diffn.* **3,** 507-513.

Prasad, G. L., Fuldner, R. A., Braverman, R., McDuffie, E. and Cooper, H. L. (1994). Expression, cytoskeletal utilization and dimer formation of tropomyosin derived from retroviral-mediated cDNA transfer. Metabolism of tropomyosin from transduced cDNA. *Eur. J. Biochem.* **224,** 1-10.

Prasad, G. L., Fuldner, R. A. and Cooper, H. L. (1993). Expression of transduced tropomyosin 1 cDNA suppresses neoplastic growth of cells transformed by the ras oncogene. *Proc. Natl. Acad. Sci. USA.* **90,** 7039-43.

Prasad, G. L., Masuelli, L., Raj, M. H. and Harindranath, N. (1999). Suppression of src-induced transformed phenotype by expression of tropomyosin-1. *Oncogene* **18,** 2027-31.

Prasad, G. L., P.S.Meissner, D.Sheer and H.L.Cooper. (1991). A cDNA encoding a muscle-type tropomyosin cloned from a human epithelial cell line: Identity with human fibroblast tropomyosin, TM1. *Biochem. Biophys. Res. Commun.* **177,** 1068-1075.

Schwartz, M. A. (1997). Integrins, oncogenes, and anchorage independence. *J. Cell Biol.* **139,** 575-8.

Sers, C., Emmenegger, U., Husmann, K., Bucher, K., Andres, A. C. and Schafer, R. (1997). Growth-inhibitory activity and downregulation of the class II tumor-suppressor gene H-rev107 in tumor cell lines and experimental tumors. *J. Cell Biol.* **136,** 935-44.

Shah, V., Bharadwaj, S., Kaibuchi, K. and Prasad, G. L. (2001). Cytoskeletal organization in tropomyosin-mediated reversion of ras-transformation: Evidence for Rho kinase. *Oncogene* **20,** 2112-2121.

Shah, V., Braverman, R. and Prasad, G. L. (1998). Suppression of Neoplastic Transformation and Regulation of Cytoskeleton by Tropomyosins. *Somatic Cell and Mol. Gen* **24,** 273-280.

Sommers, C. L. (1996). The role of cadherin-mediated adhesion in breast cancer. *J. Mammary Gland Biol. & Neoplasia* **1,** 219-29.

Yap, A. S., Brieher, W. M. and Gumbiner, B. M. (1997). Molecular and functional analysis of cadherin-based adherens junctions. *Ann. Rev. Cell Dev. Biol.* **13,** 119-46.

Figure Legends:

Figure 1. TM expression in mammary epithelial cells: A. TM expression in normal and malignant breast cells. The cell lines used are indicated. DT/TM1 and DT/TM1-TM2 cells were used as positive controls for TM expression. These are TM1-induced revertants of ras-transformed fibroblasts expressing either TM1 alone or both TM1 and TM2 (Shah *et al.*, 1998). TM2 migrates as a distinct band below TM1, which is evident in DT/TM1-TM2 cells (top panel). The cell lysates (Shah *et al.*, 2001) were probed with either a polyclonal antiserum that reacts to multiple TMs (top panel), an anti-peptide antibody that reacts to TM1 specifically (middle panel), or with anti tubulin antibody for load controls (bottom panel).

B. Northern analysis: Total cellular RNAs were probed with a full length cDNA encoding TM1 (top panel). MCF-7 and the vector control cells lack TM1 mRNA. β-actin was used for load controls (bottom panel) .Transduced TM1 is expressed as a 2.0kb mRNA, while the endogenous TM1 is synthesized as a 1.1kb RNA (Braverman *et al.*, 1996).

C. Two dimensional gel analyses of TM expression: Total cell lysates were prepared from pulse labeled MCF-7 and MCF-7/T cells and analyzed by two-dimensional gels. TM1 was identified in MCF-7/T cell sample. Cytoskeletal extracts were prepared and found that the transduced TM1 associates with the cytoskeleton (data not shown) (Prasad *et al.*, 1994).

Figure 2A. Morphology of TM1 expressing MCF-7 cells: Monolayers of MCF-7 and MCF-7/T cells were stained with H & E and photographed using an Olympus B20 microscope with 4x objective.

Figure 2B. TM1 associates with microfilaments: MCF-7 (A-C) and MCF-7/T (D-F) cells were immunostained with TM antiserum (B, E) followed by binding to FITC conjugated anti-rabbit antibody (green) and Texas red conjugated phalloidin (A, D). Merged images (C, F) are presented. The samples were viewed using a confocal microscope.

Figure 3. TM1 expression decreases growth of MCF-7 cells: Cells were cultured under normal growth medium (panel A). MCF-7 and vector control cells were cultured in charcoal stripped medium (estrogen deprivation), or in charcoal stripped serum with 100nM estradiol (panel B). The revertant MCF-7/T cell lines were cultured with or without estrogen (panel C). The revertant cells grew significantly slower than the parental or vector control MCF-7 cells in normal serum, in the absence or under estrogen supplementation.

Figure 4. TM1 suppresses anchorage independent growth: Five thousand cells were plated in soft agar as described previously (Braverman *et al.*, 1996). At the end of 3 weeks, cells were stained with nitroblue tetrazolium, photographed (A) and the number of colonies formed with each cell line were counted (B).

Figure 5. E-Cadherin-catenin complex is more tightly associated in MCF-7/T revertant cells: Cytoplasmic lysates (A) (Shah *et al.*, 2001), and RIPA lysates (Kinch *et al.*, 1995)(B) of the indicated cells were analyzed for expression of E-cadherin and the catenins by immunoblotting. Note that in the RIPA extracts, E-cadherin and the catenins are expressed at comparable levels between parental MCF-7, the vector control and the revertants.

Figure 5C. E-cadherin and β-catenin are tightly associated at the cell-cell junctions of MCF-7/T

Cells: MCF-7 (a, b) and MCF-7/T (c, d) cells were stained with either E-cadherin (a, c) or β-catenin (b, d). Multiple areas of the samples were exposed at different gate settings to accumulate different numbers of frames. E-cadherin stained samples were photographed at gate 16, and β-catenin at gate 4. Images collected at other settings are not shown. Magnification bar, 10 μm.

Figure 1.

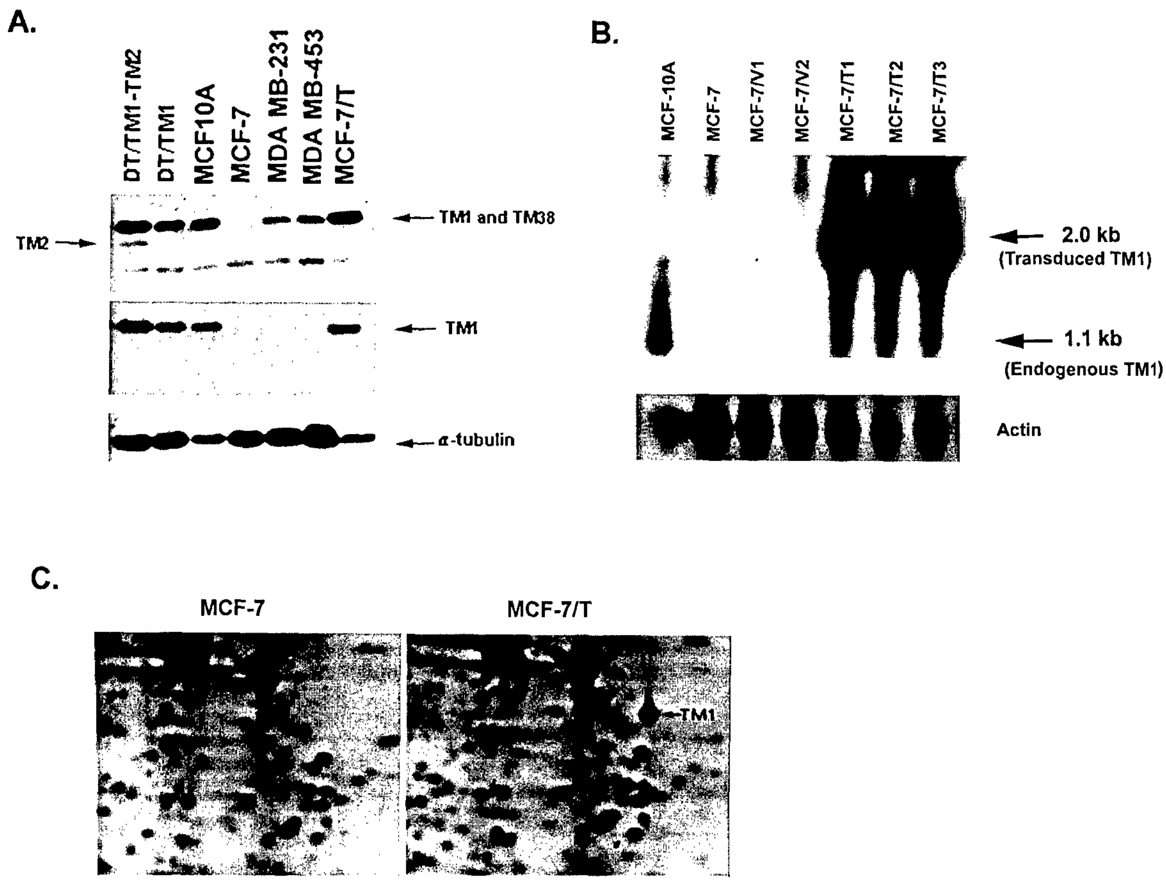

A.
DT/TM1-TM2
DT/TM1
MCF10A
MCF-7
MDA MB-231
MDA MB-453
MCF-7/T
TM2
TM1 and TM38
TM1
α-tubulin
B.
MCF-10A
MCF-7
MCF-7/V1
MCF-7/V2
MCF-7/T1
MCF-7/T2
MCF-7/T3
2.0 kb
(Transduced TM1)
1.1 kb
(Endogenous TM1)
Actin
C.
MCF-7
MCF-7/T
TM1

Figure 2

A.

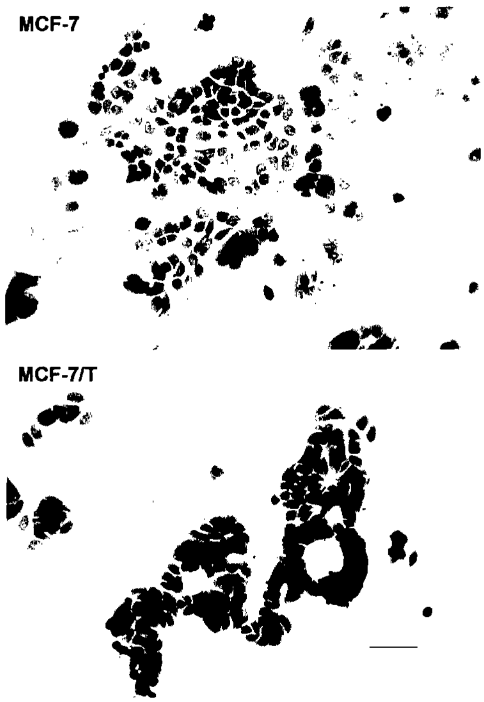


Fig. 2B.

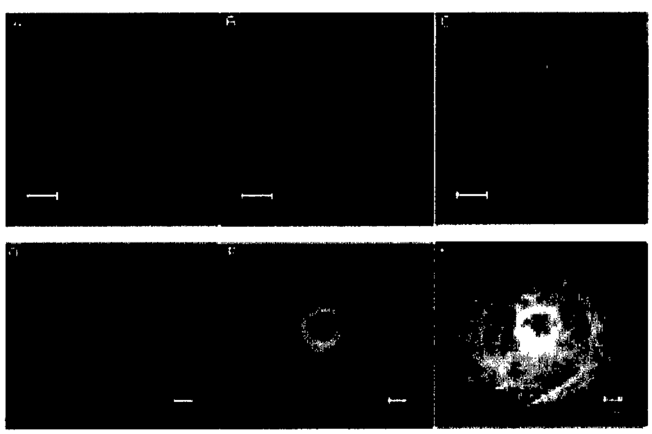


Fig. 3.

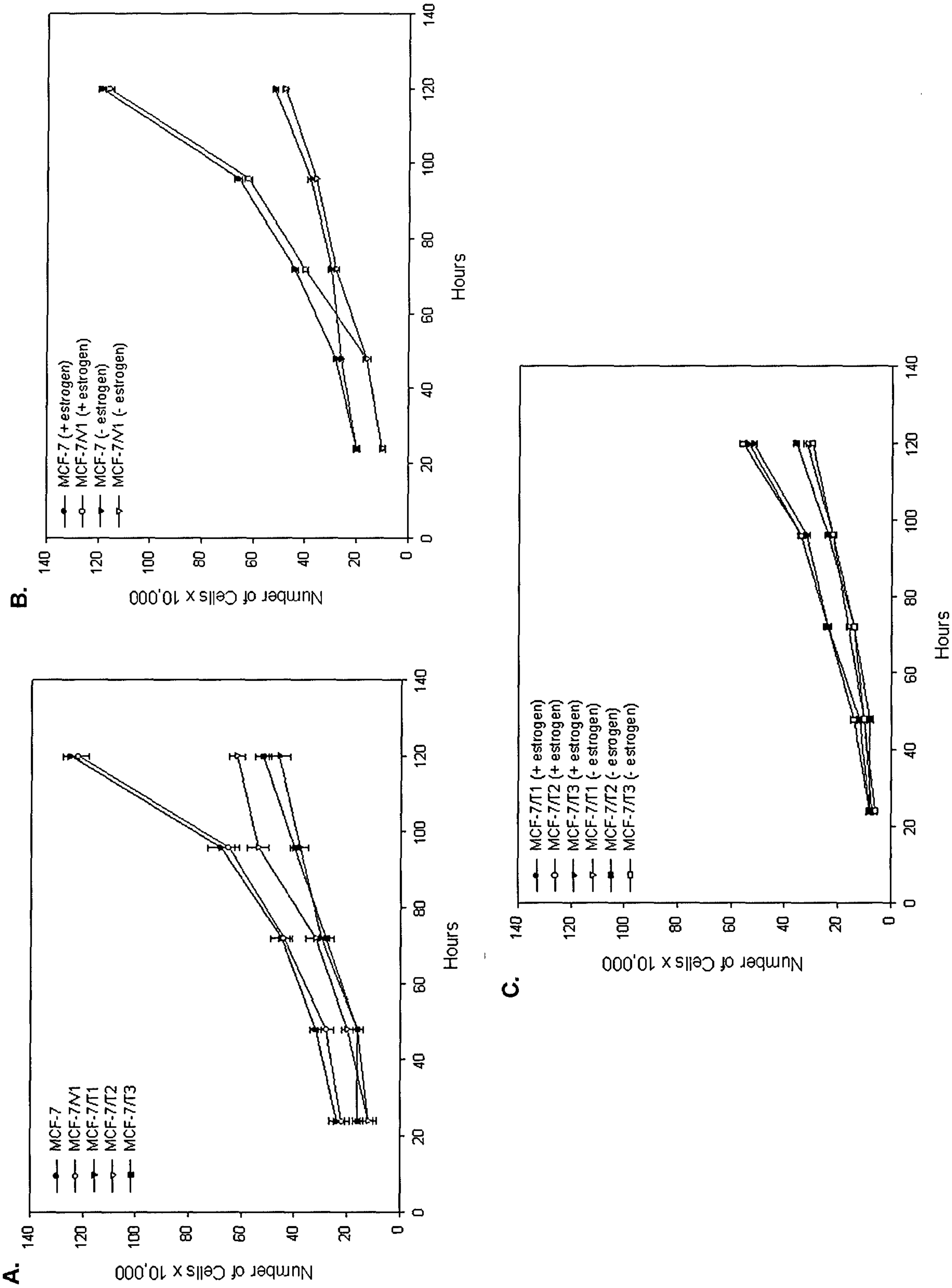
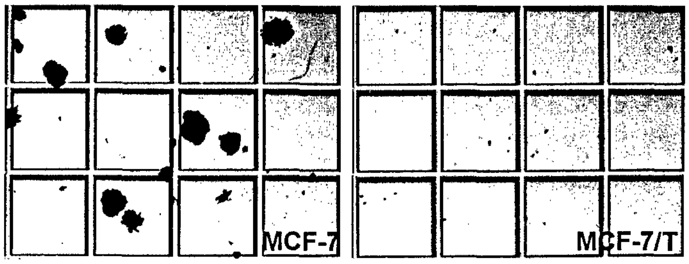

A.
MCF-7
MCF-7/V1
MCF-7/T1
MCF-7/T2
MCF-7/T3
Number of Cells x 10,000
Hours
0
20
40
60
80
100
120
140
B.
MCF-7 (+estrogen)
MCF-7/V1 (+estrogen)
MCF-7 (- estrogen)
MCF-7/V1 (- estrogen)
Number of Cells x 10,000
Hours
C.
MCF-7/T1 (+ estrogen)
MCF-7/T2 (+ estrogen)
MCF-7/T3 (+ estrogen)
MCF-7/T1 (- estrogen)
MCF-7/T2 (- esrogen)
MCF-7/T3 (- estrogen)
Number of Cells x 10,000
Hours

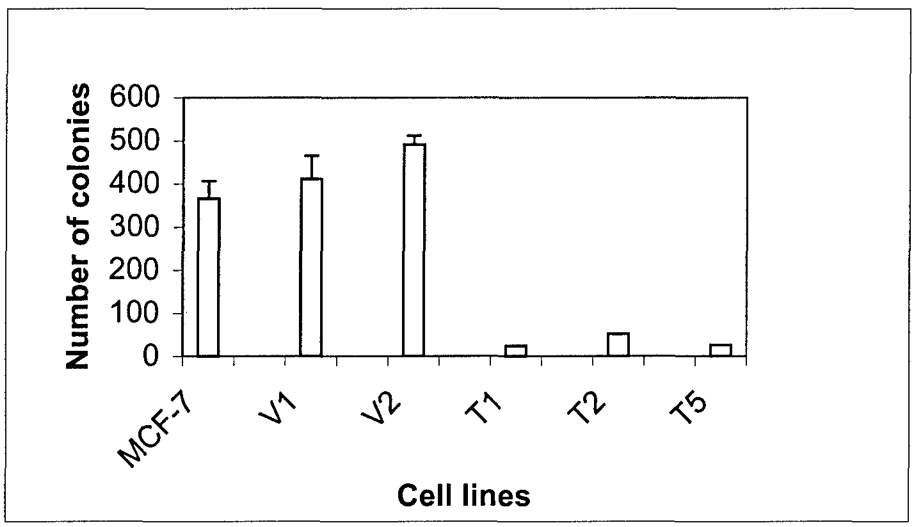
Figure 4

A.



MCF-7
MCF-7/T

B.



Number of colonies
600
500
400
300
200
100
0
MCF-7
V1
V2
T1
T2
T5
Cell lines

Figure 5.

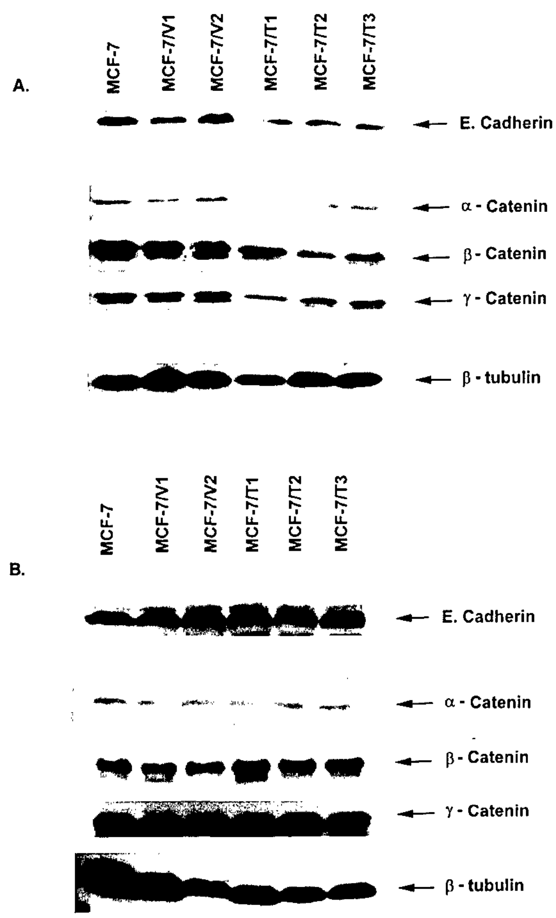
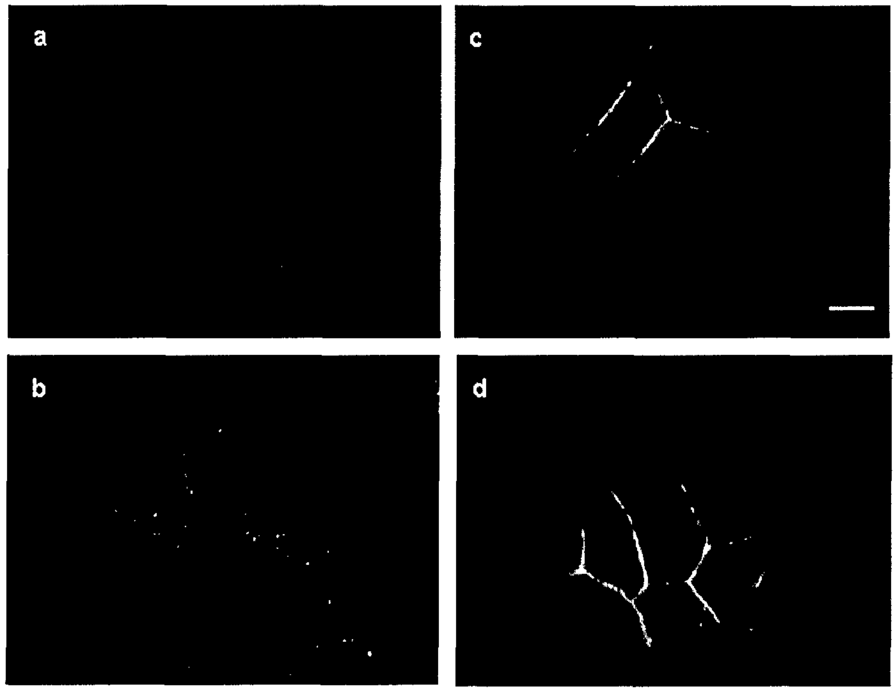

A.
MCF-7
MCF-7/V1
MCF-7/V2
MCF-7/T1
MCF-7/T2
MCF-7/T3
E. Cadherin
α - Catenin
β - Catenin
γ - Catenin
β - tubulin
B.
MCF-7
MCF-7/V1
MCF-7/V2
MCF-7/T1
MCF-7/T2
MCF-7/T3
E. Cadherin
α - Catenin
β - Catenin
γ - Catenin
β - tubulin

Figure 5C:



TROPOMYOSIN-1 IS A SUPPRESSOR OF THE MALIGNANT PHENOTYPE OF BREAST CANCER CELLS.

G. L. Prasad S. Bharadwaj, G. N. Raval, and B. K. Vonderhaar¶.
Departments of Surgery and Cancer Biology, Wake Forest University Comprehensive Cancer Center, Winston-Salem, NC 27157; and, ¶Basic Research Laboratory, Center for Cancer Research, NCI.

ABSTRACT
Dynamic regulation of cytoskeleton is crucial for the normal functioning of cells. Tropomyosins (TMs) are a family of cytoskeletal proteins that bind to and stabilize microfilaments. We are employing TMs as a paradigm to elucidate the role of cytoskeleton in tumorigenesis. Diverse oncogenic modalities suppress several key microfilament-associated proteins, such as TMs. Loss of expression of TMs results in the assembly of defective microfilaments leading to the abnormal morphology. These changes are hypothesized to contribute to the acquisition of the malignant growth phenotype. We identified that suppression of tropomyosin isoform 1 (TM1) as a common, yet pivotal biochemical event during the neoplastic transformation of mammary epithelial cells.

To investigate whether TM1 is a suppressor of breast cancer, MCF-7 cells are used as a model. Restoration of TM1 expression increases serum dependence, significantly decreases growth rates, but does not alter the estrogen sensitivity of the breast carcinoma cells. MCF-7 cells expressing TM1 grow as tighter colonies with a glandular morphology. TM1 expression abolishes the anchorage independent growth of MCF-7 cells, thus demonstrating that TM1 is a suppressor of the transformed phenotype of breast carcinoma cells. Enhanced TM1 expression results in relocalization of E-cadherin and β-catenin to cell-cell junctions, thus indicating a role for cadherin-catenin complexes in TM1-induced reversion of the malignant phenotype of breast cancer cells. These studies also suggest novel interactions between microfilaments and cell adhesion molecules in maintenance of normal growth phenotype of mammary epithelium.

Research Supported by DAMD-99-1-9395 and DAMD17-98-1-8162, and a grant from the American Cancer Society (RPG-99-069-01-CSM).